Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv
Braz. J. Vet. Parasitol., Jaboticabal, Ahead of Print, 2019
Doi: https://doi.org/10.1590/S1984-29612019031

Acanthocephalan parasites of the flounder species *Paralichthys isosceles, Paralichthys patagonicus* and *Xystreurys rasile* from Brazil

Acantocéfalos parasitos de espécies de linguados *Paralichthys isosceles, Paralichthys patagonicus* e *Xystreurys rasile* no Brasil

Michelle Cristie Gonçalves da Fonseca[1,2]; Marcelo Knoff[1]*; Nilza Nunes Felizardo[2]; Eduardo José Lopes Torres[3]; Maria Isabel Nogueira Di Azevedo[4]; Delir Corrêa Gomes[1]; Sérgio Carmona de São Clemente[2]; Alena Mayo Iñiguez[4]

[1] Laboratório de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz – FIOCRUZ, Rio de Janeiro, RJ, Brasil

[2] Laboratório de Inspeção e Tecnologia do Pescado, Universidade Federal Fluminense –UFF, Niterói, RJ, Brasil

[3] Laboratório de Helmintologia Romero Lascasas Porto, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro – UERJ, Rio de Janeiro, RJ, Brasil

[4] Laboratório de Biologia de Tripanossomatídeos – LABTRIP, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz – FIOCRUZ, Rio de Janeiro, RJ, Brasil

Received January 4, 2019
Accepted April 22, 2019

Abstract

Flounders are commercially and economically important fish. A total of 120 specimens of flounders (60 *Paralichthys isosceles*, 30 *Paralichthys patagonicus* and 30 *Xystreurys rasile*) were collected off the coast of the state of Rio de Janeiro, Brazil. The fish were measured, necropsied and filleted, and then had their organs investigated for acanthocephalans. Taxonomic identification of the parasites was based on morphological, morphometric and genetic characters. *Paralichthys isosceles* and *P. patagonicus* were parasitized by juveniles of *Serrasentis sagittifer*, *Bolbosoma turbinella*, *Corynosoma australe* and *C. cetaceum*; *Xystreurys rasile* was parasitized by *C. australe*. Genetic characterization confirmed the identification of specimens of *Bolbosoma turbinella* and *Corynosoma australe*, as demonstrated by phylogenetic analyses using both ITS and *cox*1 molecular targets. Parasite indices of prevalence, intensity, mean intensity, abundance, mean abundance, and range of infection, as well as infection site, were evaluated for each parasite species. This is the first report of *S. sagittifer* parasitizing *P. isosceles* and *P. patagonicus*, and *B. turbinella* parasitizing *P. patagonicus.*

Keywords: Acantocephalans, integrative taxonomy, *Paralichthys isosceles*, *Paralichthys patagonicus*, *Xystreurys rasile*, Brazil.

Resumo

Os linguados são peixes comercial e economicamente importantes. Um total de 120 espécimes de linguados (60 *Paralichthys isosceles*, 30 *P. patagonicus* e 30 *Xystreurys rasile*) foram coletados no litoral do estado do Rio de Janeiro, Brasil. Os peixes foram medidos, necropsiados, filetados e tiveram seus órgãos investigados para a presença de acantocéfalos. A identificação taxonômica foi baseada em caracteres morfológicos, morfométricos e genéticos. *Paralichthys isosceles* e *P. patagonicus* estavam parasitados por acantocéfalos juvenis de *Serrasentis sagittifer*, *Bolbosoma turbinella*, *Corynosoma australe* e *C. cetaceum*; *Xystreurys rasile* estava parasitado com *C. australe*. A caracterização genética confirmou a identificação dos espécimes de *Bolbosoma turbinella* e *Corynosoma australe*, como demonstrado por análises filogenéticas usando ambos marcadores moleculares ITS e *cox*1. Foram analisados os índices parasitários: prevalência, intensidade, intensidade média, abundância, abundância média, amplitude de variação da infecção e sítio de infecção de cada espécie de parasito. Este é o primeiro registro de *S. sagittifer* parasitando *P. isosceles* e *P. patagonicus*, e de *B. turbinella* parasitando *P. patagonicus.*

Palavras-chave: Acantocéfalos, taxonomia integrativa, *Paralichthys isosceles*, *Paralichthys patagonicus*, *Xystreurys rasile*, Brasil.

***Corresponding author:** Marcelo Knoff. Laboratório de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz – FIOCRUZ, Avenida Brasil, 4365, Manguinhos, CEP 21040-900, Rio de Janeiro, RJ, Brasil. e-mail: knoffm@ioc.fiocruz.br.

Introduction

Flounders (Pleuronectiformes, Paralichthyidae) are known to be ravenous predators of fish, cephalopods and crustaceans (ARAÚJO & HAIMOVICE, 2000). Species of the genera *Paralichthys* and *Xystreurys* (Paralichthyidae) occurring along the coast of the state of Rio de Janeiro, Brazil, have great commercial importance in both domestic and foreign markets (BERNARDES et al., 2005). These fish are considered to be among the so-called "fine fish" because of the high quality of their meat and their high market value (FIGUEIREDO & MENEZES, 2000; MASSA et al., 2005).

Helminth parasites have been previously reported in flounders of the family Paralichthyidae in South America, including Brazil (SANTOS et al., 2008; FELIZARDO et al., 2009a,b, 2010, 2011; ALARCOS & TIMI, 2012; FONSECA et al., 2012, 2016; KNOFF et al., 2012; ALARCOS et al., 2016).

Thorny-headed worms, or acanthocephalans, are endoparasitic helminthes. There are approximately 1290 described species (AMIN, 2013), which can be found causing various pathological conditions in marine and freshwater fish worldwide. The life-cycles of acanthocephalans involve a fish definitive host and an arthropode intermediary such as amphipod, ostracod or copepod. A few cycles also incorporate paratenic or transport hosts (WILLIAMS & JONES, 1994).

Cases of human acanthocephaliasis have been reported in association with the ingestion of raw fish, including *Acanthocephalus rauschi* Golvan, 1969 and *Corynosoma strumosum* (Rudolphi, 1802) Lühe, 1904, parasitizing Alaskan Eskimos (GOLVAN, 1969; SCHMIDT, 1971), and *Bolbosoma* Porta, 1908 and *Corynosoma* Lühe, 1904, in Japan (TADA et al., 1983; ACHA & SZYFRES, 2003; ARIZONO et al., 2012; FUJITA et al., 2016).

In Brazil, there is only one study reporting acanthocephalans of flounder *Paralichthys isosceles* Jordan, 1890 (ALARCOS et al., 2016), which motivated new surveys on the acanthocephalans parasitizing flounders species from Brazilian coast.

The aim of this study was to assess the presence of juvenile acanthocephalans parasitizing *P. isosceles*, *Paralichthys patagonicus* Jordan, 1889 and *Xystreurys rasile* (Jordan, 1891) from off the coast of the state of Rio de Janeiro, in Brazil. The acanthocephalan species encountered were identified using morphological, morphometric and genetic characters, and their parasite indices calculated and infection sites reported.

Materials and Methods

One hundred and twenty flounder specimens, 60 *P. isosceles* with mean length 33.8 cm (25.0-39.5 cm) and mean weight 420.4 g (164-680 g); 30 *P. patagonicus* with mean length 40.8 cm (28.5-59 cm) and mean weight 820.4 g (280-2530 g); and 30 *X. rasile* with mean length 24.3 cm (11.5-31 cm) and mean weight 158.5 g (20-240 g), were collected from small markets selling only fish caught offshore of the municipalities of Cabo Frio (22°52'46" S; 42°01'07" W), Niterói (22°53'00" S; 43°06'13" W), Rio de Janeiro (22°54'13" S; 43°12'35" W), and Angra dos Reis (23°00'24" S; 44°19'05" W), in the state of Rio de Janeiro, Brazil. Fish species were identified in accordance with Nakamura et al. (1986) and Figueiredo & Menezes (2000). Internal organs and musculature were examined, with all acanthocephalans found being placed in Petri dishes with 0.65% NaCl solution. Specimens were fixed in AFA (ethanol, formalin, and acetic acid), preserved in 70% ethanol, stained in Langeron's carmine, clarified in beechwood creosote, and preserved as whole mounts on Canada balsam according to Knoff & Gomes (2012). Voucher specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, state of Rio de Janeiro, Brazil.

Taxonomic classification of the parasites followed Amin (2013), while morphological identification relied on Travassos (1966), Salgado-Maldonado (1978), Measures (1992), Pereira Junior & Neves (1993) and Sardella et al. (2005). Morphometric analysis was conducted using bright-field microscopy with an Olympus BX 41 microscope. Measurements were made in millimeters and are given as ranges followed by means in parentheses. Whole-mounted samples were photographed using an Axiophot Zeiss microscope with a micrographic system, while drawings were made with the aid of a drawing tube. Some specimens were processed for scanning electron microscopy (SEM) following Lopes Torres et al. (2013), which involved fixation in 70% ethanol, dehydration in an ethanol series (70% - 100%), critical-point drying in CO_2, and coating in gold. The material was examined and photographed using a JEOL SM-25 SII SEM or a Zeiss 962 SEM, under an acceleration voltage of 15 kvolts.

Ecological terminology was according to Bush et al. (1997), as follows: prevalence, intensity, mean intensty, abundance, mean abundance, and range of infection.

Since all acanthocephalans were fixed in AFA and preserved in ethanol 70º GL, the specimens used for genetic analysis were rinsed in a 0.65% NaCl solution, identified morphologically using a stereomicroscope and preserved at -20°C until DNA extraction. *Serrasentis* sp. (n=3), *Bolbosoma* sp. (n=20), and *Corynosoma* sp. (n=8) were submitted to genetic analysis. Samples were ground under the presence of liquid nitrogen prior to DNA extraction, which was performed using a QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) (IÑIGUEZ et al., 2011, 2012). PCR was performed targeting the complete nuclear internal transcribed spacer region (ITS), including ITS-1, the 5.8S rDNA gene, and ITS-2, and the cytocrome c oxidase subunit I (*cox*1) gene. PCR primers for ITS and the conditions for amplification of ~800bp are the same as those described in Zhu et al. (1999) and Knoff et al. (2012), respectively. PCR of *cox*1 was performed targeting a 655bp fragment using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGT GACCAAAAAATCA-3'), as described in Folmer et al. (1994). Reactions were performed with a total volume of 50µl, comprising 10mM Tris–HCl (pH 8.0), 50mM KCl, 1.5mM MgCl2, 0.2mM of each dNTP, 400 ng of each primer, 1.5U Platinum *Taq* polymerase (Invitrogen), and 50 ng of genomic DNA. The PCR reactions were subjected to an initial cycle of 3min at 96º C, followed by 35 cycles at 96 °C for 1min, 56 °C for 1min, and 72 °C for 1min in a programmable thermal controller (PTC100 60v, MJ Research, Inc). PCR products were analyzed by electrophoresis, in 1.2% agarose gels for ITS and 1.8% agarose gels for cox1, and then visualized under UV light after staining with ethidium bromide. The amplicons were directly

sequenced using Big Dye Terminator v 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) in a 3100 Automated DNA Sequencer, as recommended by the suppliers. Sequencing analysis was performed using the global Basic Local Alignment Search Tool (National Center for Biotechnology Information database) and BioEdit v7.0.4.1 (Department of Microbiology, North Carolina State University, USA) (HALL, 1999). Sequences obtained in this study were compared with all acanthocephalan sequences available in GenBank for both targets. ITS datasets were constructed with all reference sequences available in Genbank (10/2018) for the genera *Bolbosoma*, *Corynosoma* and *Polymorphus*. The *cox*1 dataset contained all available sequences in Genbank (10/2018) for the genera *Bolbosoma* and *Corynosoma*. *Hexaglandula corynosoma* was used as an outgroup. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 7 (TAMURA et al., 2013). Phylogenetic trees were constructed using the Neighbor-joining (NJ) method, the Kimura-2-Parameters (K2P) model, and following the CBOL barcoding protocol (http://www.barcodeoflife.org/content/ resources/ standards-and-guidelines). The pairwise deletion parameter was applied with 1000 bootstrap replicates. Maximum likelihood (ML) trees were constructed using the best-fitting model of DNA substitution, as determined by the Bayesian information criterion (BIC).

Results

A total of 134 juvenile acanthocephalans were collected: 47 from *P. isosceles*, 81 from *P. patagonicus* and 6 from *X. rasile*. *Paralichthys isosceles* and *P. patagonicus* were parasitized by a species of *Serrasentis,* a species of *Bolbosoma*, and two species of *Corynosoma*. *Xystreurys rasile* was parasitized by a species of *Corynosoma*. The prevalence of acanthocephalans found in each species of flounders was: 48.3% for *P. isosceles*, 40.0% for *P. patagonicus*, and 10.0% for *X. rasile*.

Some juveniles were alive and exhibited limited motility. Acanthocephalans were taxonomically identified as below.

Rhadinorhynchidae Lühe, 1912

Serrasentis Van Cleave, 1923

Serrasentis sagittifer (Linton, 1889) Van Cleave, 1923 (Figures. 1-3)

Features observed in 19 juveniles, 8 from *P. isosceles,* and 11 from *P. patagonicus*. Body elongate and cylindrical. Anterior extremity with hooks forming longitudinally-curved U-shaped combs on

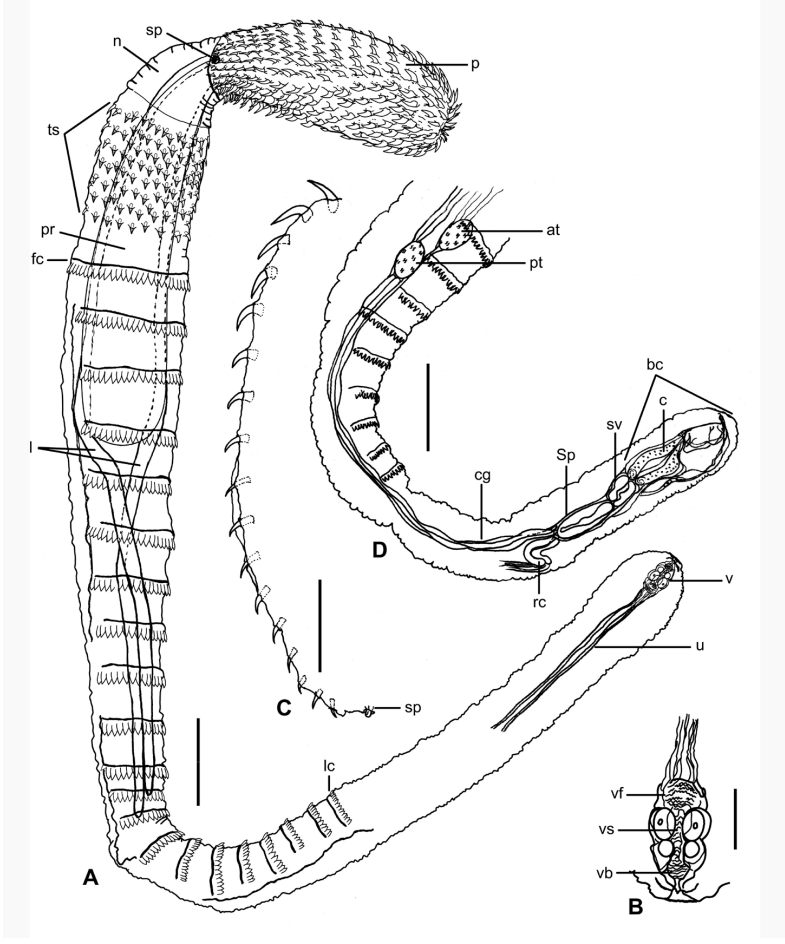

Figure 1. Juveniles of *Serrasentis sagittifer* in *Paralichthys isosceles*. A: entire female in lateral view; B: detail of vagina in ventral view; C: hooks of proboscis and sensory papilla; D: posterior portion of male in lateral view. Abreviations: at = anterior testis, bc = bursa copulatrix, c = cirrus, cg = cement glands, fc = first comb, l = leminisci, lc = last comb, n = neck, p = proboscis, pr = proboscis receptacle, pt = posterior testis, rc = receptacle cement, sp = sensory papilla, Sp = Saefftigen's pouch, sv = seminal vesicle, ts = trunk spines, u = uterus, v = vagina, vb = vagina bulb, vf = vagina funnel, vs = vagina sphincther. Bars A and D = 0.4 mm, B = 0.1 mm, and C = 0.2 mm.

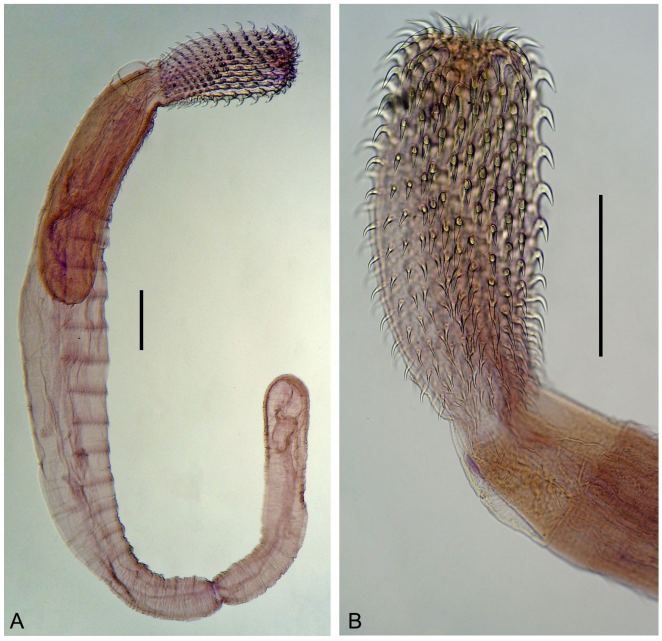

Figure 2. Juvenile of *Serrasentis sagittifer* in *Paralichthys patagonicus*. A: entire male in lateral view; B: detail of proboscis and neck. Bars A and B = 0.4 mm.

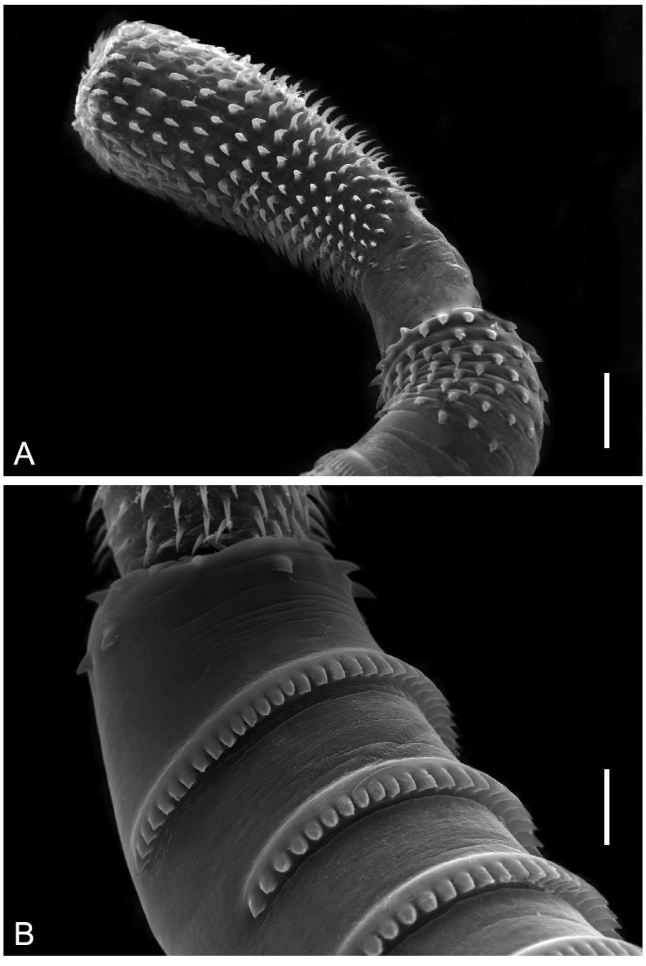

Figure 3. Juveniles of *Serrasentis sagittifer* in *Paralichthys isosceles* under SEM. A: proboscis, neck and anterior trunk spines. B: detail of trunk combs. Bars: A = 200 µm and B = 100 µm.

the ventral and lateral surfaces. Proboscis claviform, armed with 22-24 longitudinal hook rows each with 16-17 hooks. Neck short, conical. Proboscis hooks thicker ventrally than dorsally, decreasing in size from apex to base. Proboscis receptacle with thick double wall. Pair of extremely long leminisci extend for half the length of the trunk. Small sensory papilla present on each side of the base of proboscis. Trunk elongate, with 24-28 longitudinal rows of 8-9 spines on anterior extremity, followed posteriorly by transversal rows of combs of spines (18-20 in males and 18-22 in females) occupying half of the length of the trunk on the ventral surface. Male genital apparatus: two ovoid testes in tandem; four tubular cement glands; cement receptacle linked to elongate Saefftigen's pouch; pyriform seminal vesicle; and spiny cirrus inside bursa copulatrix. Female genital apparatus: ovarium balls; uterine bell; uterus straight; and muscular vagina divided into funnel, sphincter, and bulb.

Morphometric parameters are shown in Table 1.

Hosts*: P. isosceles* and *P. patagonicus*.

Parasitic indices for *P. isosceles* (Pi): prevalence = 20%, mean intensity = 1.67 ± 0.77, mean abundance = 0.33 ± 1.41 and range of infection = 1-3 and for *P. patagonicus* (Pp): prevalence = 26.7%, mean intensity = 4.37 ± 3.25, mean abundance = 1.17 ± 4.94 and range of infection = 1-11.

Infection sites for *P. isosceles:* intestine and for *P. patagonicus:* stomach and intestine.

Collected specimens for *P. isosceles*: 20 and for *P. patagonicus*: 35.

Deposited specimens: CHIOC 37828, 37829, 37830, 37831(Pi); CHIOC 38079a, b, 38080, 38081a-c, 38082, 38083 (Pp).

Polymorphidae Meyer, 1931

Bolbosoma Porta, 1908

Bolbosoma turbinella (Diesing, 1851) (Figures. 4-6)

Features observed in 12 juveniles, 8 from *P. isosceles,* and 4 from *P. patagonicus*. Body elongate, anterior region dilated, proboscis cylindrical and armed with 20-22 longitudinal rows of 7-8 hooks each. Neck short, wider at posterior end, without hooks. Bulb flattened with trunk spines arranged in 38-40 longitudinal rows with 21-22 hooks each, extending dorsally and ventrally covering about half of the proboscis receptacle; spines smaller near base of neck. Pair of slightly convoluted leminisci of similar length present. Male genital apparatus: testes ovoid, in line; four tubular cement glands followed by elongate Saefftigen's pouch; seminal vesicle ovoid; and cirrus inside bursa copulatrix. Female genital apparatus: ovarian balls; uterine bell elongate; uterus straight; and muscular vagina divided into funnel, sphincter, and bulb.

Morphometric parameters are shown in Table 2.

Hosts: *P. isosceles* and *P. patagonicus*.

Parasitic indices for *P. isosceles* (Pi): prevalence = 13.3%, mean intensity = 2.00 ± 1.21, mean abundance = 0.27 ± 2.12 and range of infection = 1-4 and for *P. patagonicus* (Pp): prevalence = 13.3%, mean intensity = 2.50 ± 1.29, mean abundance = 0.33 ± 2.12 and range of infection = 1-4.

Infection sites for *P. isosceles*: stomach and intestine and for *P. patagonicus*: intestine.

Collected specimens for *P. isosceles*: 16 and for *P. patagonicus*: 10.

Deposited specimens: CHIOC 37832, 37833, 37834, 37835 (Pi); CHIOC 38084, 37988, 37989, 37990 (Pp).

Corynosoma Lühe, 1904

Table 1. Morphometric data of juveniles of *Serrasentis sagittifer* collected from *Paralichthys isosceles* and *Paralichthys patagonicus* in the state of Rio de Janeiro, Brazil.

Parameters	*Paralichthys isosceles*		*Paralichthys patagonicus*	
	Males (n = 4)	Females (n = 4)	Males (n = 7)	Females (n = 4)
Body (L)	3.8-7.9 (6.5)	6.24-7.46 (6.85)	4.77-7.52 (5.92)	4.35-9.2 (6.76)
Trunk (L)	3.12-7.05 (5.70)	6.0-7.0 (6.50)	3.94-6.67 (4.98)	3.05-8.17 (5.53)
Trunk (W)	0.25-0.72 (0.54)	0.50-0.66 (0.58)	0.52-0.57 (0.55)	0.40-0.65 (0.52)
Proboscis (L)	0,67-0.97 (0.81)	1.10-1.12 (1.11)	0.52-0.95 (0.72)	0.75-1.02 (0.92)
Proboscis (W)	0.20-0.27 (0.22)	0.30-0.45 (0.37)	0.27-0.40 (0.31)	0.22-0.40 (0.32)
Basal hooks (L)	0.037-0.050 (0.041)	0.045-0.055 (0.050)	0.042-0.052 (0.049)	0.045-0.055 (0.050)
Basal hooks (W)	0.007-0.010 (0.008)	0.007-0.010 (0.008)	0.010-0.012 (0.011)	0.007-0.010 (0.008)
Subapical hooks (L)	0.055-0.072 (0.063)	0.067-0.095 (0.078)	0.072-0.080 (0.075)	0.067-0.095 (0.077)
Subapical hooks (W)	0.017-0.020 (0.018)	0.015-0.025 (0.020)	0.012-0.027 (0.016)	0.015-0.025 (0.020)
Apical hooks (L)	0.062-0.087 (0.076)	0.090-0.100 (0.095)	0.072-0.080 (0.076)	0.080-0.097 (0.086)
Apical hooks (W)	0.017-0.027 (0.021)	0.015-0.030 (0.022)	0.016-0.027 (0.020)	0.015-0.030 (0.021)
Neck (L)	0.20-0.37 (0.30)	0.24-0.46 (0.35)	0.30-0.35 (0.32)	0.24-0.46 (0.33)
Neck (W)	0.20-0.32 (0.25)	0.25-0.48 (0.36)	0.22-0.37 (0.30)	0.27-0.35 (0.32)
Proboscis receptacle (L)	1.10-1.80 (1.38)	1.52-1.74 (1.63)	1.55-1.77 (1.65)	1.13-1.93 (1.57)
Proboscis receptacle (W)	0.22-0.32 (0.28)	0.36-0.40 (0.38)	0.35-0.37 (0.36)	0.18-0.47 (0.27)
Trunk spines (L)	0.035-0.067 (0.054)	0.31-0.55 (0.43)	0.057-0.062 (0.059)	0.052-0.062 (0.059)
Trunk spines (W)	0.017-0.030 (0.024)	0.027-0.030 (0.029)	0.027-0.037 (0.031)	0.012-0.037 (0.022)
Leminisci (L)	2.50-3.20 (2.90)	3.20-4.25 (3.90)	2.25-2.82 (2.50)	1.92-2.97 (2.07)
Anterior testis (L)	0.10-0.25 (0.16)	-	0.10-0.14 (0.12)	-
Anterior testis (W)	0.05-0.12 (0.08)	-	0.08-0.11 (0.09)	-
Posterior testis (L)	0.10-0.21 (0.15)	-	0.09-0.13 (0.10)	-
Posterior testis (W)	0.05-0.10 (0.07)	-	0.07-0.11 (0.09)	-
Cement glands	0.15-0.25 (0.19)	-	0.10-0.20 (0.15)	
Uterine bell (L)	-	0.12-0.16 (0.14)	-	0.06-0.12 (0.09)
Uterine bell (W)	-	0.03-0.04 (0.035)	-	0.035-0.070 (0.052)
Uterus (L)	-	0.46-0.52 (0.48)	-	0.40-0.62 (0.50)
Vagina (L)	-	0.18-0.20 (0.19)	-	0.13-0.18 (0.15)

Measurements are in millimeters, means in parentheses. L= length; W= width; n = number of larvae measured.

Corynosoma australe Johnston, 1937 (Figures. 7-8)

Features observed in 14 juveniles, 1 from *P. isosceles,* 11 from *P. patagonicus* and 2 from *X. rasile*. Body pyriform, anterior region dilated and proboscis cylindrical. Proboscis wider at posterior end, armed with 18 longitudinal rows of 12-14 hooks each; 9-11 anterior hooks with well developed posteriorly directed roots and three small basal hooks with small anteriorly directed roots. Neck short, wider at posterior end. Anterior portion of trunk swollen and flattened in form of a bulb. Proboscis receptacle double-walled. Tegumental spines covering bulb dorsally at fore-trunk, extending to the ventral surface at the posterior end. Pair of moderately short, lobe-like leminisci reaching half the length of proboscis receptacle. In some female specimens, genital and tegumental spines are contiguous in the ventral region, but clearly distinguishable. Genital opening surrounded by triangular genital spines that are larger than tegumental spines. Male genital apparatus: testes rounded, contiguous; six claviform cement glands, followed by elongate Saefftigen's pouch; elongate seminal vesicle; and bursa copulatrix. Female genital apparatus: ovarian balls; uterine bell elongate; uterus straight; and muscular vagina divided into funnel, sphincter, and bulb. Male genital spines show a distinctive radial distribution pattern with 14 spines on each side of the opening of the bursa copulatrix, and one central spine, called "c", on the dorsal surface. Some spines are bifid, such as observed in 1 and 1'. Female genital spines are located on the ventral side and are smaller than those of males.

Morphometric parameters are shown in Table 3.

Hosts: *P. isosceles*, *P. patagonicus* and *X. rasile*.

Parasitic indices for *P. isosceles* (Pi): prevalence = 5%, mean intensity = 3.33 ± 1.52, mean abundance = 0.17 ± 2.82, range of infection = 1-5; for *P. patagonicus* (Pp)*:* prevalence = 16.7%, mean intensity = 3.60 ± 2.56, mean abundance = 0.60 ± 3.53 and range of infection = 1-7 and for *X. rasile* (Xr): prevalence = 10%, mean intensity = 2.00 ± 0.74, mean abundance = 0.20 ± 1.41 and range of infection = 1-4.

Infection sites for *P. isosceles*: stomach and intestine; and for *P. patagonicus* and *X. rasile*: intestine.

Collected specimens for *P. isosceles*: 10, for *P. patagonicus*: 18 and for *X. rasile:* 6.

Deposited specimens: CHIOC 37836b (Pi); CHIOC 37172, 37837, 37964, 38048a-b, 38049a-b, 38050a-c (Pp); 38055, 38056 (Xr).

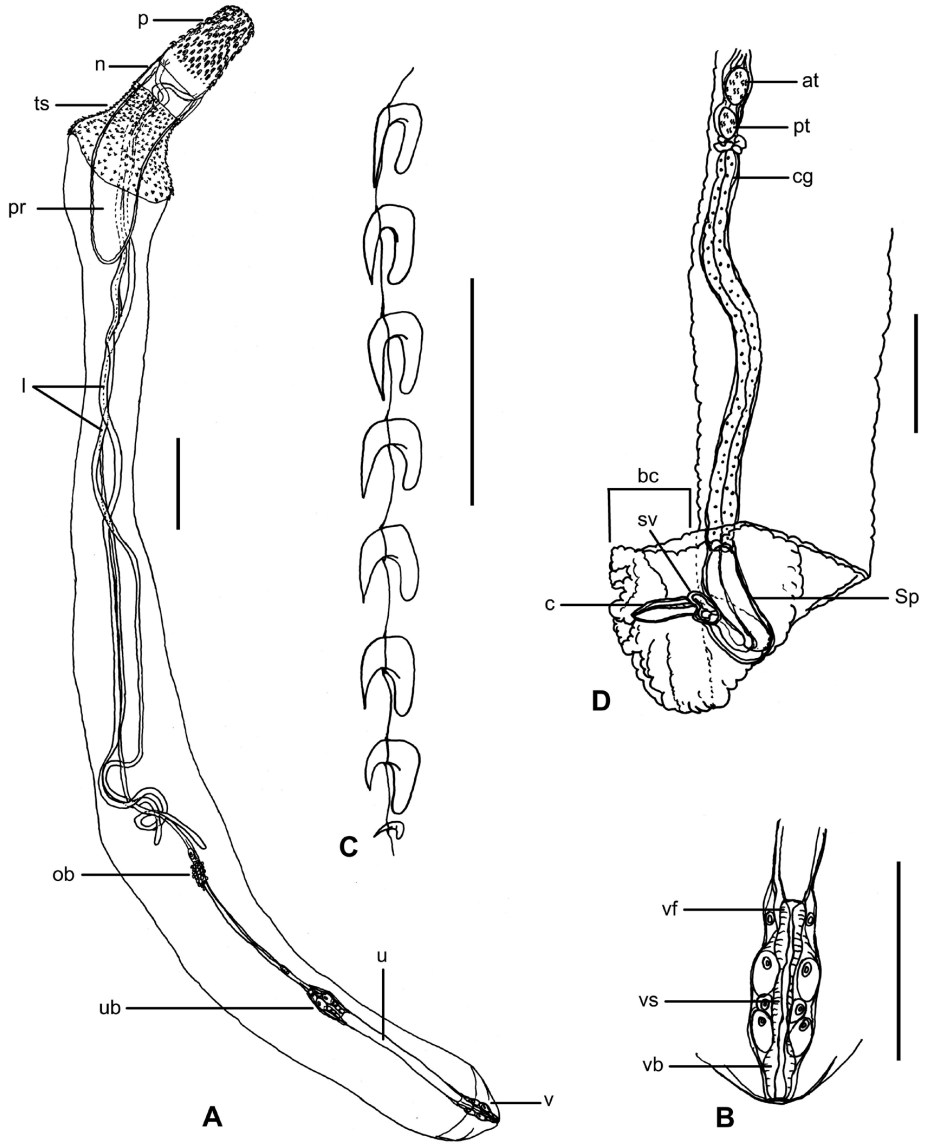

Figure 4. Juveniles of *Bolbosoma turbinella* in *Paralichthys isosceles*. A: entire female in lateral view; B: detail of vagina in ventral view; C: hooks of proboscis; D: posterior portion of male in lateral view. Abreviations: at = anterior testis, bc = bursa copulatrix, c = cirrus, cg = cement glands, l = leminisci, n = neck, ob = ovarian balls, p = proboscis, pr = proboscis receptacle, pt = posterior testis, Sp = Saefftigen's pouch, sv = seminal vesicle, ts = trunk spines, u = uterus, ub = uterine bell, v = vagina, vb = vagina bulb, vf = vagina funnel, vs = vagina sphincter. Bars A = 0.8 mm, B and D = 0.4 mm, and C = 0.2 mm.

Corynosoma cetaceum (Johnston & Best, 1942) (Figure 9)

Features observed in 13 juveniles, 1 from *P. isosceles,* and 12 from *P. patagonicus*. Body pyriform, anterior region dilated and proboscis cylindrical. Proboscis wider at posterior end, armed with 18-20 longitudinal rows of 15-16 hooks each; 12-13 anterior hooks with well-developed posteriorly-directed roots and 2-3 (usually 2) small basal hooks with small anteriorly directed roots. Neck short, wider at posterior end. Anterior portion of trunk swollen and flattened in form of a bulb. In females, fore-trunk and hind-trunk delimited by a typical ventral fold; in males, fold delimiting fore-trunk and hind-trunk is more superficial and not always discernible. Proboscis receptacle double-walled. Tegumental spines covering bulb dorsally for the length of the fore-trunk; in males, these spines extend ventrally covering 60-75% of trunk length; in females, these spines extend ventrally covering 97-98% of trunk length, and the trunk possesses two ventral transverse folds, that are devoid of spines, and delimit a blunt lobe between fore- and hind-trunk. A pair of moderately short, lobe-like leminisci extend over half of the proboscis receptacle. Genital spines absent in both sexes; less frequently males possess a unique, clearly distinguishable genital spine. Male genital apparatus: testes round, contiguous; six claviform cement glands, followed by elongate Saefftigen's pouch; seminal vesicle elongate; and bursa copulatrix. Female genital apparatus: ovarian balls; uterine bell elongate; uterus straight; and a muscular vagina divided into funnel, sphincter, and bulb.

Morphometric parameters are shown in Table 4.

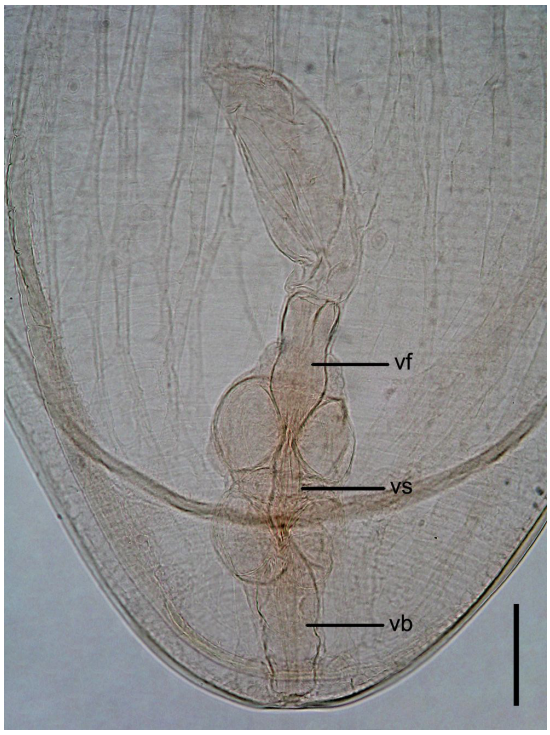

Figure 5. Juvenile female of *Bolbosoma turbinella* in *Paralichthys patagonicus*. Detail of vagina in ventral view. Abreviations: vb = vagina bulb, vf = vagina funnel, vs = vagina sphincter. Bar = 0.1 mm.

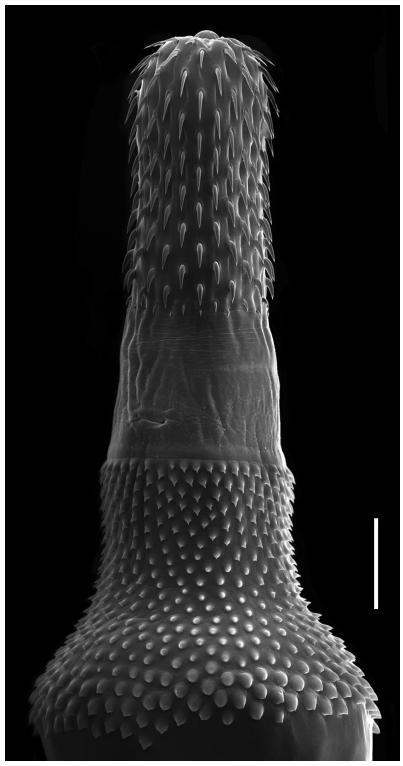

Figure 6. Juvenile of *Bolbosoma turbinella* in *Paralichthys isosceles* under SEM. Proboscis, neck and trunk spines. Bar = 200 µm.

Table 2. Morphometric data of juveniles of *Bolbosoma turbinella* collected from *Paralichthys isosceles* and *Paralichthys patagonicus* in the state of Rio de Janeiro, Brazil.

Parameters	*Paralichthys isosceles*		*Paralichthys patagonicus*
	Males (n = 4)	Females (n = 4)	Females (n = 4)
Body (L)	6.45-8.05 (7.05)	7.05-11.62 (8.49)	6.25-11.00 (8.56)
Trunk (L)	4.62-6.07 (5.16)	5.35-9.00 (6.51)	4.72-8.72 (6.65)
Trunk (W)	0.25-0.70 (0.59)	0.47-0.85 (0.65)	0.72-0.95 (0.85)
Proboscis (L)	0.53-0.61 (0.58)	0.42-0.80 (0.60)	0.40-0.75 (0.55)
Proboscis (W)	0.21-0.27 (0.23)	0.26-0.47 (0.33)	0.26-0.28 (0.27)
Basal hooks (L)	0.025-0.035 (0.030)	0.022-0.027 (0.024)	0.020-0.030 (0.025)
Basal hooks (W)	0.010-0.012 (0.011)	0.007-0.012 (0.010)	0.007-0.010 (0.009)
Subapical hooks (L)	0.072-0.080 (0.075)	0.070-0.080 (0.073)	0.067-0.082 (0.075)
Subapical hooks (W)	0.017-0.022 (0.019)	0.017-0.025 (0.021)	0.010-0.022 (0.016)
Apical hooks (L)	0.075-0.082 (0.079)	0.070-0.092 (0.081)	0.062-0.092 (0.078)
Apical hooks (W)	0.012-0.015 (0.013)	0.012-0.017 (0.015)	0.010-0.015 (0.013)
Neck (L)	0.15-0.19 (0.17)	0.14-0.25 (0.18)	0.18-0.26 (0.21)
Neck (W)	0.28-040 (0.34)	0.30-0.53 (0.40)	0.42-0.55 (0.49)
Proboscis receptacle (L)	0.92-1.50 (1.11)	0.78-1.76 (1.18)	0.59-1.54 (1.16)
Proboscis receptacle (W)	0.29-0.37 (0.33)	0.21-0.43 (0.33)	0.41-0.48 (0.43)
Trunk spines (L)	0.037-0.052 (0.044)	0.037-0.047 (0.041)	0.040-0.050 (0.044)
Trunk spines (W)	0.022-0.030 (0.027)	0.015-0.032 (0.025)	0.015-0.027 (0.022)
Leminisci (L)	3.75-5.10 (4.24)	3.87-7.12 (5.04)	2.55-7.0 (4.41)
Anterior testis (L)	0.120-0.135 (0.124)	-	-
Anterior testis (W)	0.050-0.095 (0.072)	-	-
Posterior testis (L)	0.090-0.145 (0.117)	-	-
Posterior testis (W)	0.060-0.095 (0.073)	-	-
Uterine bell (L)	-	0.20-0.42 (0.33)	0.36-0.47 (0.39)
Uterine bell (W)	-	0.08-0.14 (0.12)	0.10-0.13 (0.12)
Uterus (L)	-	0.65-1.24 (0.92)	0.62-1.00 (0.83)
Vagina (L)	-	0.36-0.41 (0.38)	0.30-0.43 (0.36)

Measurements are in millimeters, means in parentheses. L= length; W= width; n = number of larvae measured.

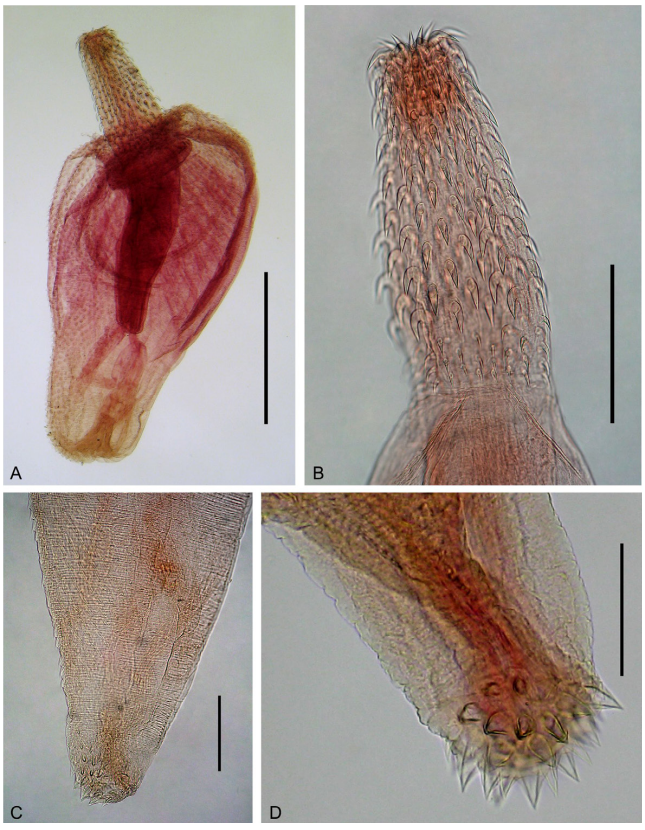

Figure 7. Juveniles of *Corynosoma australe* in *Paralichthys patagonicus*. A: entire female in lateral view; B: detail of female proboscis; C: posterior end of female in lateral view; D: posterior end of male in ventral view. Bars A = 1 mm, B = 0.25 mm, C = 0.2 mm, and D = 0.1 mm.

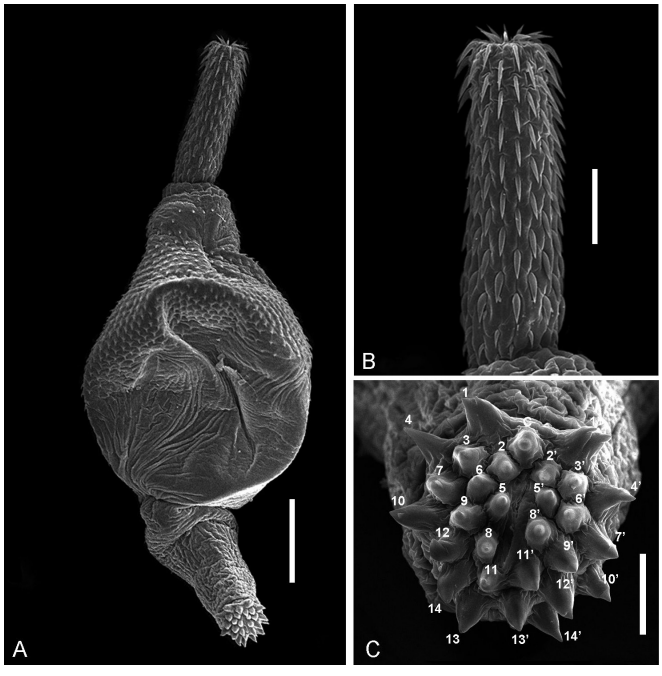

Figure 8. Juvenile of *Corynosoma australe* in *Paralichthys patagonicus* under SEM. A: entire male in dorsolateral view; B: detail of male proboscis in dorsolateral view; C: posterior portion of male with detail of genital spines in dorsolateral view. Bars A = 200 µm, B = 100 µm, C = 40 µm.

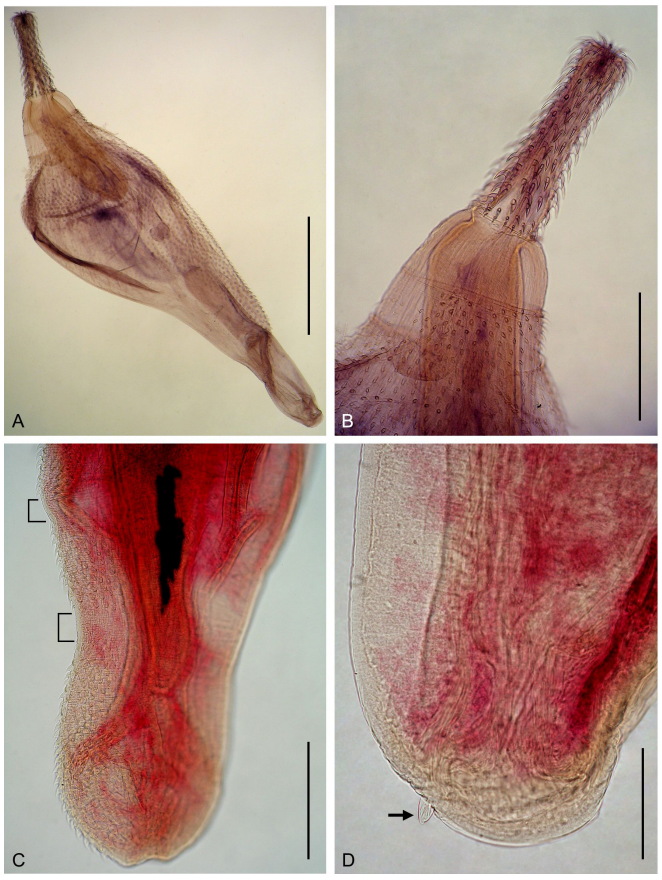

Figure 9. Juveniles of *Corynosoma cetaceum* in *Paralichthys patagonicus*. A: entire male in lateral view; B: proboscis, neck and trunk spines in lateral view; C: posterior end of female in lateral view. Brackets indicate the two ventral trunk folds that are devoid of spines, in lateral view; D: posterior end of male with detail of genital spine in lateral view. Bars A = 1 mm, B = 0.5 mm, C = 0.4 mm, and D = 0.1 mm.

Hosts: *P. isosceles* and *P. patagonicus*.

Parasitic indices for *P. isosceles* (Pi): prevalece = 1.67%, intensity = 1.00, abundance = 0.02 and for *P. patagonicus* (Pp): prevalence = 26.7%, mean intensity = 2.25 ± 1.76, mean abundance = 0.60 ± 5.65 and range of infection = 1-6.

Infection sites for *P. isosceles* and *P. patagonicus*: intestine.

Collected specimens for *P. isosceles*: 1 and for *P. patagonicus*: 18.

Deposited specimens: CHIOC 37836a (Pi); CHIOC 38051, 38052a-f, 38053, 38054 (Pp).

Genetic analysis. Of the acanthocephalan specimens submitted to genetic analysis, four specimens of *Bolbosoma* sp. and one specimen of *Corynosoma* sp. yielded DNA sequences: three specimens of *Bolbosoma* sp. and the one of *Corynosoma* sp. for the ITS region and two of *Bolbosoma* sp. and the one of *Corynosoma* sp. for the *cox*1 gene.

An ITS dataset (843bp) was constructed with all the reference sequences available for the genera *Bolbosoma*, *Corynosoma* and *Polymorphus*, and the *Bolbosoma* sp. samples HE14, HE23, and HE25 (ITS Dataset I). In order to include the short sequence recovered from *Corynosoma* sp. (the HE38 sample; 574pb) in the ITS phylogenetic analysis, the

Table 3. Morphometric data of juveniles of *Corynosoma australe* collected from *Paralichthys isosceles*, *Paralichthys patagonicus* and *Xystreurys rasile* in the state of Rio de Janeiro, Brazil.

Parameters	*Paralichthys isosceles*	*Paralichthys patagonicus*		*Xystreurys rasile*
	Males (n = 1)	Males (n = 4)	Females (n = 7)	Males (n = 2)
Body (L)	2.90	1.77-2.42 (2.15)	2.82-3.6 (2.98)	2.07-3.32 (2.70)
Trunk (L)	2.20	1.42-1.57 (1.51)	1.7-2.6 (2.05)	1.45-2.70 (2.07)
Trunk (W)	0.92	0.54-0.6 (0.56)	0.68-1.36 (1.01)	0.55-0.85 (0.70)
Proboscis (L)	0.63	0.34-0.47 (0.42)	0.04-0.90 (0.62)	0.56-0.76 (0.66)
Proboscis (W)	0.19	0.14-0.17 (0.15)	0.17-0.38 (0.27)	0.10-0.37 (0.23)
Basal hooks (L)	0.017-0.027 (0.021)	0.015-0.027 (0.019)	0.025-0.040 (0.030)	0.015-0.022 (0.018)
Basal hooks (W)	0.005-0.010 (0.008)	0.007-0.010 (0.009)	0.005-0.01 (0.006)	0.005-0.007 (0.006)
Subapical hooks (L)	0.045-0.055 (0.050)	0.042-0.050 (0.044)	0.045-0.075 (0.059)	0.040-0.070 (0.050)
Subapical hooks (W)	0.010-0.015 (0.011)	0.010-0.015 (0.011)	0.01-0.012 (0.011)	0.010-0.012 (0.011)
Apical hooks (L)	0.045-0.050 (0.047)	0.040-0.050 (0.045)	0.045-0.080 (0.061)	0.047-0.072 (0.06)
Apical hooks (W)	0.007-0.012 (0.009)	0.007-0.010 (0.008)	0.007-0.01 (0.008)	0.010-0.012 (0.011)
Neck (L)	0.24	0.12-0.16 (0.14)	0.16-0.29 (0.22)	0.11-0.13 (0.12)
Neck (W)	0.33	0.20-0.26 (0.23)	0.27-0.61 (0.39)	0.24-0.33 (0.28)
Proboscis receptacle (L)	0.91	0.62-0.75 (0.67)	0.64-1.24 (1.00)	0.67-1.07 (0.87)
Proboscis receptacle (W)	0.18	0.12-0.17 (0.15)	0.21-0.30 (0.27)	0.23-0.26 (0.24)
Trunk spines (L)	0.040-0.045 (0.041)	0.032-0.045 (0.040)	0.025-0.045 (0.035)	0.035-0.055 (0.045)
Trunk spines (W)	0.017-0.022 (0.019)	0.012-0.017 (0.015)	0.010-0.022 (0.016)	0.007-0.025 (0.016)
Genital spine (L)	0.042-0.045 (0.043)	0.042-0.045 (0.044)	0.025-0.047 (0.037)	0.035-0.045 (0.040)
Genital spine (W)	0.017-0.022 (0.019)	0.015-0.022 (0.018)	0.012-0.040 (0.026)	0.015-0.025 (0.020)
Leminisci (L)	0.70	0.49	0.31-0.70 (0.54)	0.43-0.53 (0.48)
Leminisci (W)	0.24	0.26	0.21-0.28 (0.24)	0.26-0.32 (0.29)
Right testis (L)	0.13	0.07-0.10 (0.09)	-	0.04-0.13 (0.09)
Right testis (W)	0.085	0.055-0.085 (0.07)	-	0.100-0.105 (0.102)
Left testis (L)	0.11	0.065-0.095 (0.085)	-	0.11-0.14 (0.12)
Left testis (W)	0.010	0.045-0.060 (0.053)	-	0.110-0.130 (0.120)
Cement glands (L)	0.12-0.16 (0.14)	0.09-0.12 (0.11)	-	0.12-0.15 (0.14)
Cement glands (W)	0.025-0.030 (0.027)	0.025-0.030 (0.028)	-	0.025-0.035 (0.030)
Uterus (L)	-	-	0.17-0.30 (0.24)	-
Vagina (L)	-	-	0.19-0.28 (0.22)	-

Measurements are in millimeters, means in parentheses. L= length; W= width; n = number of larvae measured.

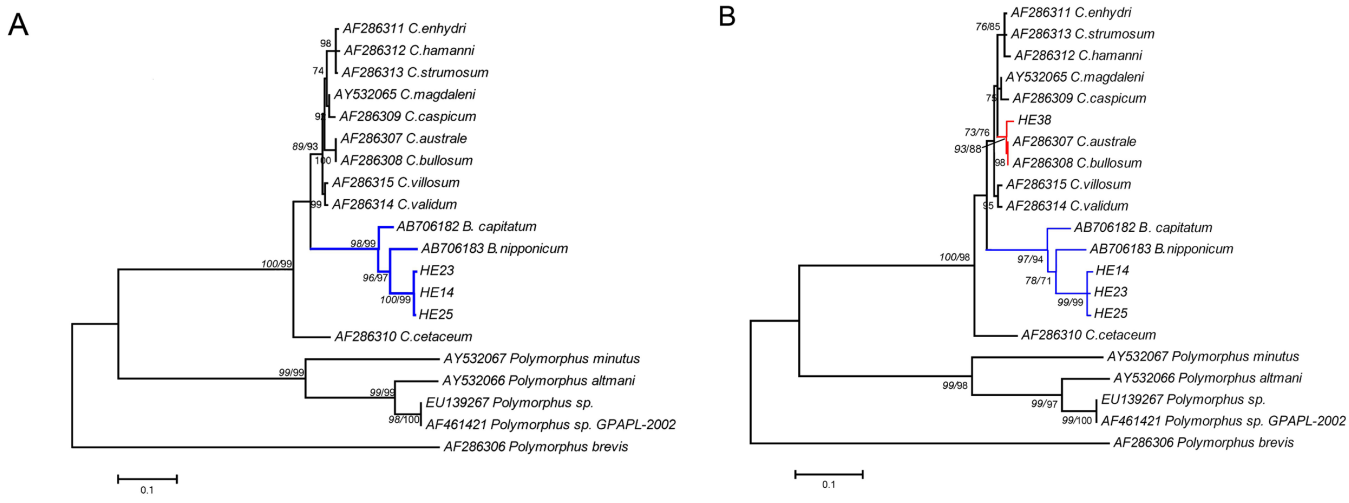

Figure 10. Maximum likelihood (ML) phylogenetic analyses of ITS sequence data. A: ITS Dataset I including sequences HE14, HE23 and HE25 from this study and GenBank reference sequences (species and accession numbers are shown) using the TN92+G model; B: ITS Dataset II including sequence HE14, HE23, HE25, HE38 from this study and GenBank reference sequences using the K2P+G model. Numbers at nodes are bootstrap support values, when higher than 50%. Regular numbers correspond to ML while italicized numbers correspond to Neighbor-joining bootstraps. Bars represent the expected number of substitutions per nucleotide.

Table 4. Morphometric data of juveniles of *Corynosoma cetaceum*, collected from *Paralichthys isosceles* and *Paralichthys patagonicus* in the state of Rio de Janeiro, Brazil.

Parameters	*Paralichthys isosceles*	*Paralichthys patagonicus*	
	Female (n = 1)	Males (n = 7)	Females (n = 5)
Body (L)	3.53	2.93-5.24 (4.19)	2.43-3.34 (3.07)
Trunk (L)	2.7	2.55-3.82 (3.24)	2.25-2.45 (2.36)
Trunk (W)	0.97	1.00-1.58 (1.25)	0.76-1.05 (0.91)
Proboscis (L)	0.77	0.72-1.07 (0.83)	0.54-0.92 (0.81)
Proboscis (W)	0.26	0.24-0.36 (0.29)	0.27-0.34 (0.29)
Basal hooks (L)	0.030-0.040 (0.035)	0.030-0.040 (0.036)	0.027-0.042 (0.034)
Basal hooks (W)	0.007-0.010 (0.008)	0.007-0.012 (0.009)	0.005-0.010 (0.008)
Subapical hooks (L)	0.072-0.090 (0.079)	0.065-0.085 (0.075)	0.072-0.087 (0.082)
Subapical hooks (W)	0.015-0.020 (0.017)	0.015-0.020 (0.017)	0.012-0.022 (0.017)
Apical hooks (L)	0.075-0.087 (0.082)	0.065-0.082 (0.074)	0.080-0.090 (0.085)
Apical hooks (W)	0.012-0.015 (0.013)	0.012-0.015 (0.013)	0.010-0.017 (0.013)
Neck (L)	0.23	0.17-0.33 (0.22)	0.15-0.23 (0.17)
Neck (W)	0.51	0.35-0.55 (0.44)	0.29-0.64 (0.41)
Proboscis receptacle (L)	1.13	1.00-1.37 (0.001)	1.13-1.30 (1.18)
Proboscis receptacle (W)	0.38	0.23-0.40 (0.33)	0.32-0.42 (0.36)
Trunk spines (L)	0.042-0.050 (0.045)	0.050-0.057 (0.052)	0.040-0.052 (0.045)
Trunk spines (W)	0.037-0.040 (0.040)	0.015-0.037 (0.024)	0.027-0.042 (0.036)
Leminisci (L)	-	0.40-0.84 (0.62)	0.32-0.54 (0.50)
Leminisci (W)	-	0.22-0.48 (0.31)	0.28-0.44 (0.35)
Right testis (L)	-	0.12-0.18 (0.15)	-
Right testis (W)	-	0.06-0.12 (0.08)	-
Left testis (L)	-	0.10-0.17 (0.13)	-
Left testis (L)	-	0.09-0.12 (0.10)	-
Cement glands (L)	-	0.21-0.33 (0.28)	-
Cement glands (W)	-	0.04-0.06 (0.05)	-
Copulatrix bursa (L)	-	0.36-1.03 (0.56)	-
Copulatrix bursa (W)	-	0.18-0.42 (0.28)	-
Uterus (L)	0.54	-	0.45-0.58 (0.51)
Vagina (L)	0.28	-	0.24-0.30 (0.26)

Measurements are in millimeters, means in parentheses. L= length; W= width; n = number of larvae measured.

dataset was shortened and named ITS Dataset II. The *cox*1 dataset contained reference sequences and the sequences from *Bolbosoma* sp. and *Corynosoma* sp. (samples HE25, HE38 and HE43). The best-fitting model of DNA substitution using BIC was the Tamura 3-parameter model (TN92) with the gamma distribution (+G) for ITS Dataset I; K2P+G for ITS Dataset II; and Hasegawa-Kishino-Yano model (HKY) plus gamma distribution and invariable sites (+G+I) for the *cox*1 dataset. Nucleotide sequences were deposited in GenBank with the accession numbers KU314817-KU314823.

Using the ITS Dataset I, phylogenetic trees based on NJ K2P and ML TN92+G (Figure 10A) confirmed the identification of *Bolbosoma* sp. The three specimens grouped with the two available ITS sequences for *Bolbosoma capitatum* and *Bolbosa nipponicum* with high bootstrap values (98% and 99%, for NJ and ML analyses, respectively). Using the ITS Dataset II (NJ K2P and ML K2P), one specimen (HE38) of *Corynosoma* sp. (Figure 10B), was included in the genus-specific cluster (NJ = 73%, ML = 76%), as closely related to the *C. australe* and *C. bullosum* subcluster (NJ = 88%, ML = 93%). Both datasets, and both mehtods used, agreed in showing the genera *Bolbosoma* and *Corynosoma* as monophyletic and closely related (NJ = 100%, ML = 99/98%). The only exception was when the ML method was applied since *C. cetaceum* (AF286310) did not group within the *Corynosoma* genus cluster, but instead was placed as an outgroup basal to *Bolbosoma* + *Corynosoma*. The phylogenetic trees produced by NJ K2P and ML HKY+G+I using the *cox*1 data set revealed the same topology (Figure 11). The specimen HE38 grouped within the *C. australe* cluster with maximun bootstrap values for both NJ and ML. In addition, two *Bolbosoma* sp. sequences (HE25 and HE43) clustered with the two *Bolbosoma* sp. *cox*1 sequences available (NJ = 94%, ML= 97%), and robustly with *B. turbinella* with maximun bootstrap value (NJ/ML = 100%) (Figure 11).

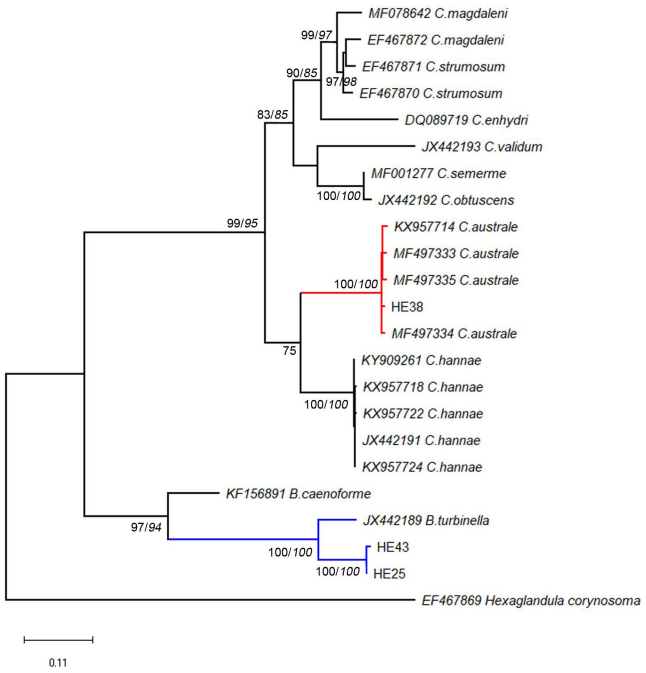

Figure 11. Maximum likelihood (ML) phylogenetic analysis of *cox*1 gene sequences, including sequences HE25, HE38 and HE43 from this study and GenBank reference sequences. Numbers at nodes are bootstrap support values, when higher than 50%. Regular numbers correspond to ML analysis with the HKY model and G+I parameters, while italicized numbers correspond to Neighbor-joining analysis with the K2P model. Bar represents the expected number of substitutions per nucleotide.

Discussion

The acanthocephans identified as *S. sagittifer* in the present study, morphologically resemble those previously recorded as immature and adult specimens of this parasite from several teleost species from various regions of the world (AL-ZUBAIDY & MHAISEN, 2012; ABDEL-GHAFFAR et al., 2014; MOHAMADAIN & ADEL, 2015). The difference in size found by the present study is likely due to the developmental stage of the parasite, while the differences found in the number of combs is likely due to intraspecific variation, as reported by Al-Zubaidy & Mhaisen (2012). Therefore, as only juvenile specimens of *S. sagittifer* were found in the present study, the flounders are acting as paratenic hosts.

The juvenile of *B. turbinella* collected in the present study were identified according to previous morphological descriptions of adults collected from cetaceans, and mainly involved characters relating to proboscis hooks and the distribution of trunk spines (MEASURES, 1992). Morphometrically, these specimens were smaller then those of adult collected from *Balaenoptera borealis* off the coast of Rio de Janeiro, Brazil, by Machado Filho (1964), which can be explained by them being of different ontogenetic stages.

The morphological and morphometric characters of the *C. australe* specimens collected in the present study are in accordance with previous descriptions reported for specimens from Brazilian and Argentinean fish hosts (KNOFF et al., 2001; SARDELLA et al., 2005; AZNAR et al., 2016). According to Sardella et al. (2005), the male genital opening is surrounded by three irregular rows of 18-34 triangular spines, while the specimens of the present study had 29 spines distributed around the male genital opening (Figure 8).

The specimens of *C. cetaceum* of the present study exhibited morphological and morphometric characters in agreement with the original descriptions, as well as other descriptive studies (AZNAR et al., 2002, 2016; SARDELLA et al., 2005).

Alarcos & Timi (2012) reported juvenile acanthocephalans from the same hosts as investigated in the present study, but from Argentina, and found only the species *C. australe* and *C. cetaceum*. In comparison with our results, *C. australe* of that study had higher parasite indices in *P. patagonicus* with prevalence of 94.12% and mean abundance of 6.35, in *P. isosceles* with prevalence of 92.16% and mean abundance of 14.69 and in *X. rasile* with prevalence of 89.58% and mean abundance of 9.23. Alarcos & Timi (2012) also found *C. cetaceum* occurring in *P. patagonicus* with higher parasite indices (prevalence of 74.51% and mean abundance of 2.55). Furthermore, they found it present in *X. rasile*. Subsequently, Alarcos et al. (2016) reported juvenile acanthocephalans parasitizing *P. isosceles* in Rio de Janeiro, Brazil. In contrast to our results, these authors only found two acanthocephalan species: *C. australe* with similar parasite indices (prevalence of 7.9% and mean abundance of 0.1), and *B. turbinella* with higher indices for hosts collected in Cabo Frio (prevalence of 36.8% and mean abundance of 0.5) and in Niterói, state of Rio de Janeiro (prevalence of 25% and mean abundance of 0.6). The present study differed by having found the additional acanthocephalan species *S. sagittifer* and *C. cetaceum* in *P. isosceles*.

According to Spalding et al. (2007), marine ecoregions have relatively homogeneous species compositions that are clearly distinct from adjacent systems. Species composition is likely determined by the predominance of a small number of ecosystems and/or a distinct suite of oceanographic or topographic features. The dominant biogeographic agents that define ecoregions vary from location to location but may include physico-chemical and biological agents. In ecological terms, these ecoregions are strongly cohesive units that are sufficiently large to encompass ecological or life history processes for most sedentary species. Although some marine ecoregions may have important levels of endemism, this is not a key determinant in ecoregion identification, as it has been for terrestrial ecoregions. Therefore, features observed among and within ecoregions can influence their fish parasite communities, and thus explain the parasite compositions of the flounder species from waters off Necochea, Argentina, by Alarcos & Timi (2012), and from waters off Rio de Janeiro, Brazil, by Alarcos et al. (2016) and the present study.

Serrasentis sagittifer and *C. cetaceum* were recently reported from paralichthyid fish at higher prevalences than those found in the present study. Barton & Smales (2015) investigated six species of the genus *Pseudorhombus* Bleeker, 1862, from Australian waters, and found cystacants of *S.* cf. *sagittifer* (prevalecne of 36.32%) in fish from the western Gulf of Carpentaria, and *S.* cf. *sagittifer* (prevalence of 25%) and *Corynosoma cetaceum* (prevalence of 21.25%) in fish off the central coast of Queensland, showing that these acanthocephalan species occur in other paralichthyid fish in other parts of the world. Thus, *P. isosceles* and *P. patagonicus*

are new host records for *S. sagittifer*, and *P. patagonicus* is a new host record for *B. turbinella*.

The parasitization of paralichthyid fish by species of *Corynosoma* and *Bolbosoma turbinella* recorded in the present study indicates that these fish are positioned at an intermediate trophic level of the marine food web where they act as paratenic hosts, as has been reported for other species of this family (FUJITA et al., 2016), while marine mammals and birds are final hosts (HERNÁNDEZ-ORTS et al., 2017).

The species of *Corynosoma* and *Bolbosoma turbinella* were found alive in the present study, which reinforces the importance of hygienic-sanitary practices because some species of these two genera are involved with zoonoses. This fact was reported by Fujita et al. (2016), who also commented that such infections are closely associated with eating uncooked food, and are mostly reported from Japan because of the traditional food culture there (i.e., sushi and sashimi). Even though the polymorphid acanthocephalan specimens of the present study were not found in the musculature, they can migrate there via inadequated fish cleanning, with the rupture of the walls of the intestine and stomach, and stay available for ingestion, potentially infecting consumers. As suggested by FAO/WHO (2014) and Fujita et al. (2016), treatments of heating or freezing are desired for the prevention of parasite infections by these species, as is the usual case for other food-borne parasites, and recently in Brazil (PORTO ALEGRE, 2016), the minimum requirements for the production, preparation and commercialization of sushis and sashimis in the city of Porto Alegre (Brazil) were published. Therefore, future studies should be conducted to evaluate the zoonotic potential of the polymorphid species analyzed in the present study.

Acknowledgements

The authors would like to thank Heloisa Nogueira Diniz and Ricardo Baptista Schmidt (Serviço de Produção e Tratamento de Imagens do Instituto Oswaldo Cruz/FIOCRUZ) for processing the figures. This work was supported by CNPq fellowships (AMI: 307932/2014-1 and 312934/2017-3; SCSC: 308048/2013-0; MCGF: 140093/2012-5 and 150140/2018-5); a FAPERJ grant (AMI: 26/202.945/2016) and a CAPES grant (MINDA: ECM09/2009).

References

Abdel-Ghaffar F, Morsy K, Abdel-Gaber R, Mehlhorn H, Al Quraishy S, Mohammed S. Prevalence, morphology, and molecular analysis of *Serrasentis sagittifer* (Acanthocephala: Palaeacanthocephala: Rhadinorhynchidae), a parasite of gilthead Sea bream *Sparus aurata* (Sparidae). *Parasitol Res* 2014; 113(7): 2445-2454. http://dx.doi.org/10.1007/s00436-014-3889-6. PMid:24828344.

Acha PN, Szyfres B. *Zoonoses and communicable diseases common to man and animals*. 3rd ed. Washington: PAHO; 2003. (Scientific and Technical Publication; no. 580; Parasitoses; vol. III).

Alarcos AJ, Pereira AN, Taborda NL, Luque JL, Timi JT. Parasitological evidence of stocks of *Paralichthys isosceles* (Pleuronectiformes: Paralichthyidae) at small and geographical scales in South American Atlantic coasts. *Fish Res* 2016; 173(3): 221-228. http://dx.doi.org/10.1016/j.fishres.2015.07.018.

Alarcos AJ, Timi JT. Parasite communities in three sympatric flounder species (Pleuronectiformes: Paralichthyidae): similar ecological filters driving toward repeatable assemblages. *Parasitol Res* 2012; 110(6): 2155-2166. http://dx.doi.org/10.1007/s00436-011-2741-5. PMid:22167375.

Al-Zubaidy AB, Mhaisen FT. A record of two species of Acanthocephala (Echinorhynchida: Rhadinorhynchidae) from Red Sea fishes, Yemeni coastal waters. *Mesop J Mar Sci* 2012; 27(1): 15-28.

Amin OM. Classification of the Acanthocephala. *Folia Parasitol (Praha)* 2013; 60(4): 273-305. http://dx.doi.org/10.14411/fp.2013.031. PMid:24261131.

Araújo JN, Haimovice M. Determinação de idades e crescimento do linguado branco *Paralichthys patagonicus* (Jordan, 1889) no sul do Brasil. *Rev Bras Oceanogr* 2000; 48(1): 61-70. http://dx.doi.org/10.1590/S1413-77392000000100005.

Arizono N, Kuramochi T, Kagei N. Molecular and histological identification of the acanthocephalan *Bolbosoma* cf. *capitatum* from the human small intestine. *Parasitol Int* 2012; 61(4): 715-718. http://dx.doi.org/10.1016/j.parint.2012.05.011. PMid:22634485.

Aznar FJ, Berón-Vera B, Crespo EA, Raga JA. Presence of genital spines in a male *Corynosoma cetaceum* Johnston and Best, 1942 (Acanthocephala). *J Parasitol* 2002; 88(2): 403-404. http://dx.doi.org/10.1645/0022-3395(2002)088[0403:POGSIA]2.0.CO;2. PMid:12054020.

Aznar FJ, Crespo EA, Raga JA, Hernández-Orts J. Trunk spines in cystacanths and adults of *Corynosoma* spp. (Acanthocephala): *Corynosoma cetaceum* as an exceptional case of phenotypic variability. *Zoomorphology* 2016; 135(1): 19-31. http://dx.doi.org/10.1007/s00435-015-0290-7.

Barton DP, Smales LR. Acanthocephalan cystacanths from flatfish (Order Pleuronectiformes) in Tropical Australian Waters. *J Parasitol* 2015; 101(4): 429-435. http://dx.doi.org/10.1645/15-731.1. PMid:25807200.

Bernardes RA, Figueiredo JL, Rodrigues AR, Fischer LG, Vooren CM, Haimovici M, et al. *Peixes da zona econômica exclusiva da região sudeste-sul do Brasil: Levantamento com armadilhas, pargueiras e rede de arrasto de fundo*. São Paulo: Editora da Universidade de São Paulo; 2005.

Bush AO, Lafferty K, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* 1997; 83(4): 575-583. http://dx.doi.org/10.2307/3284227. PMid:9267395.

Felizardo NN, Justo MC, Knoff M, Fonseca MCG, Pinto RM, Gomes DC. Juvenile didymozoids of the types, Torticaecum and Neotorticaecum (Didymozoidae: Digenea), from new marine fish hosts (Pisces: Teleostei) in the Neotropical region of Brazil. *J Helminthol* 2011; 85(3): 270-275. http://dx.doi.org/10.1017/S0022149X10000507. PMid:20854704.

Felizardo NN, Knoff M, Pinto RM, Gomes DC. Larval anisakid nematodes of the flounder, *Paralichthys isosceles* Jordan, 1890 (Pisces: Teleostei) from Brazil. *Neotrop Helminthol* 2009a; 3(2): 57-64.

Felizardo NN, Menezes RC, Tortelly R, Knoff M, Pinto RM, Gomes DC. Larvae of *Hysterothylacium* sp. (Nematoda: Anisakidae) in the sole fish *Paralichthys isosceles* Jordan, 1890 (Pisces: Teleostei) from the littoral of the state of Rio de Janeiro, Brazil. *Vet Parasitol* 2009b; 166(1-2): 175-177. http://dx.doi.org/10.1016/j.vetpar.2009.08.004. PMid:19713041.

Felizardo NN, Torres EJL, Fonseca MCG, Pinto RM, Gomes DC, Knoff M. Cestodes of the flounder *Paralichthys isosceles* Jordan, 1890 (Osteichthyes - Paralichthyidae) from the state of Rio de Janeiro, Brazil. *Neotrop Helminthol* 2010; 4(2): 113-126.

Figueiredo JL, Menezes NA. *Manual de peixes marinhos do Sudeste do Brasil*. São Paulo: Universidade de São Paulo, Museu de Zoologia; 2000. (VI Teleostei; no. 5).

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 1994; 3(5): 294-299. PMid:7881515.

Fonseca MC, Knoff M, Felizardo NN, Di Azevedo MI, Torres EJ, Gomes DC, et al. Integrative taxonomy of Anisakidae and Raphidascarididae (Nematoda) in *Paralichthys patagonicus* and *Xystreurys rasile* (Pisces: Teleostei) from Brazil. *Int J Food Microbiol* 2016; 235(20): 113-124. http://dx.doi.org/10.1016/j.ijfoodmicro.2016.07.026. PMid:27491056.

Fonseca MCG, São Clemente SC, Felizardo NN, Gomes DC, Knoff M. Trypanorhyncha cestodes of hygienic-sanitary importance infecting flounders *Paralichthys patagonicus* Jordan, 1889 and *Xystreurys rasile* (Jordan, 1891) of the Neotropical region, Brazil. *Parasitol Res* 2012; 111(2): 865-874. http://dx.doi.org/10.1007/s00436-012-2912-z. PMid:22488201.

Food and Agriculture Organization – FAO, World Health Organisation – WHO. *Multicriteria-bases ranking for risk management of food-borne parasites*. Geneva: WHO Press; 2014. (Microbiological Risk Assessment Series; no. 23).

Fujita T, Waga E, Kitaoka K, Imagawa T, Komatsu Y, Takanashi K, et al. Human infection by acanthocephalan parasites belonging to the genus *Corynosoma* found from small bowel endoscopy. *Parasitol Int* 2016; 65(5 PtA): 491-493. http://dx.doi.org/10.1016/j.parint.2016.07.002. PMid:27396515.

Golvan YJ. Systematique des Acanthocephales (Acanthocephala Rudolphi, 1801). L'ordre des Paleacanthocephala Meyer, 1931. La super-familie des Echinorhynchoidea (Cobbold, 1876) Golvan et Houin, 1963. *Mem Mus Natl Hist Nat Paris Ser A Zool* 1969; 57(single): 1-373.

Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999; 41: 95-98.

Hernández-Orts JS, Brandão M, Georgieva S, Raga JA, Crespo EA, Luque JL, et al. From mammals back to birds: Host-switch of the acanthocephalan *Corynosoma australe* from pinnipeds to the Magellanic penguin *Spheniscus magellanicus. PLoS One* 2017; 12(10): e0183809. http://dx.doi.org/10.1371/journal.pone.0183809. PMid:28981550.

Iñiguez AM, Carvalho VL, Motta MR, Pinheiro DC, Vicente AC. Genetic analysis of *Anisakis typica* (Nematoda: Anisakidae) from cetaceans of the northeast coast of Brazil: new data on its definitive hosts. *Vet Parasitol* 2011; 178(3-4): 293-299. http://dx.doi.org/10.1016/j.vetpar.2011.01.001. PMid:21324600.

Iñiguez AM, Leles D, Jaeger LH, Carvalho-Costa FA, Araújo A, Amazonas Research Group. Genetic characterisation and molecular epidemiology of *Ascaris* spp. from humans and pigs in Brazil. *Trans R Soc Trop Med Hyg* 2012; 106(10): 604-612. http://dx.doi.org/10.1016/j.trstmh.2012.06.009. PMid:22944771.

Knoff M, Felizardo NN, Iñiguez AM, Maldonado A Jr, Torres EJ, Pinto RM, et al. Genetic and morphological characterisation of a new species of the genus *Hysterothylacium* (Nematoda) from *Paralichthys isosceles* Jordan, 1890 (Pisces: Teleostei) of the Neotropical Region, State of Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz* 2012; 107(2): 186-193. http://dx.doi.org/10.1590/S0074-02762012000200006. PMid:22415256.

Knoff M, Gomes DC. Metodologia básica para coleta e o processamento de helmintos parasitos. In: Molinaro EM, Caputo LFG, Amendoeira MRR, editores. *Conceitos e métodos para a formação de profissionais em laboratórios de saúde*. vol. 5. Rio de Janeiro: EPSJV; 2012. p. 251-281.

Knoff M, São Clemente SC, Pinto RM, Gomes DC. Digenea and Acanthocephala of elasmobranch fishes from the Southern Coast of Brazil. *Mem Inst Oswaldo Cruz* 2001; 96(8): 1095-1101. http://dx.doi.org/10.1590/S0074-02762001000800012. PMid:11784929.

Lopes Torres EJ, Souza W, Miranda K. Comparative analysis of *Trichuris muris* surface using conventional, low vacuum, environmental and field emission scanning electron microscopy. *Vet Parasitol* 2013; 196(3-4): 409-416. http://dx.doi.org/10.1016/j.vetpar.2013.02.026. PMid:23537947.

Machado Filho DA. Contribuição para o conhecimento do gênero "Bolbosoma" Porta, 1908 (Palaeacanthocephala, Polymorphidae). *Rev Bras Biol* 1964; 24(3): 341-348. PMid:14275360.

Massa AE, Palacios DL, Paredi ME, Crupkin M. Postmortem changes in quality indices of ice-stored flounder (*Paralichthys patagonicus*). *J Food Biochem* 2005; 29(5): 570-590. http://dx.doi.org/10.1111/j.1745-4514.2005.00050.x.

Measures LN. *Bolbosoma turbinella* (Acanthocephala) in a blue whale, *Balaenoptera musculus*, stranded in the St. Lawrence Estuary, Quebec. *J Helminthol Soc Wash* 1992; 59(2): 206-211.

Mohamadain HS, Adel A. Light and scanning electron microscopy on *Serrasentis sagittifer* Linton, 1889 (Acanthocephala): Palaeacanthocephala: Rhadinorhynchidae) infecting the common sea bream in Egypt. *J Egypt Soc Parasitol* 2015; 45(1): 23-28. http://dx.doi.org/10.12816/0010846. PMid:26012215.

Nakamura I, Inada T, Takeda M, Hatanaka H. *Important fishes trawled off Patagonia*. Tokyo: Japan Marine Fishery Resource Research Center; 1986.

Pereira Junior J, Neves LFM. *Corynosoma australe* Johnston, 1937 (Acanthocephala, Polymorphidae) em *Micropogonias furnieri* (Desmarest, 1823) (Perciformes, Sciaenidae) do litoral do Rio Grande do Sul. *Comun Mus Cien PUCRS Ser Zool* 1993; 6: 51-61.

Porto Alegre. Portaria SMS nº 1109 de 23/08/2016. Norma Municipal - Porto Alegre – RS. Aprova as exigências mínimas para produção, preparo e comercialização de sushis e sashimis no Município de Porto Alegre. *Normas Brasil* [online], ago. 2016 [cited 2019 March 13]. Available from: http://www.normasbrasil.com.br/norma/ portaria-1109-2016-porto-alegre-rs_327789.html

Salgado-Maldonado G. Acantocéfalos de peces V. Redescripción de cuatro especies de palaeacantocéfalos parásitos de peces de México. *An Inst Biol Univ Nal Autón* México Ser *Zool* 1978; 49(1): 49-70.

Santos CP, Gibson DI, Tavares LER, Luque JL. Checklist of acanthocephala associated with the fishes of Brazil. *Zootaxa* 2008; 1938: 1-22.

Sardella NH, Mattiucci S, Timi JT, Bastida RO, Rodríguez DH, Nascetti G. *Corynosoma australe* Johnston, 1937 and *C. cetaceum* Johnston & Best, 1942 (Acanthocephala: Polymorphidae) from marine mammals and fishes in Argentinian waters: allozyme markers and taxonomic status. *Syst Parasitol* 2005; 61(2): 143-156. http://dx.doi.org/10.1007/s11230-005-3131-0. PMid:15980967.

Schmidt GD. Acanthocephalan infection of man, with two new records. *J Parasitol* 1971; 57(3): 582-584. http://dx.doi.org/10.2307/3277920. PMid:5090967.

Spalding MD, Fox HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, et al. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *Bioscience* 2007; 57(7): 573-583. http://dx.doi.org/10.1641/B570707.

Tada I, Otsuji Y, Kamiya H, Mimori T, Sakaguchi Y, Makizumi S. The first case of a human infected with an acanthocephalan parasite, *Bolbosoma* sp. *J Parasitol* 1983; 69(1): 205-208. http://dx.doi.org/10.2307/3281300. PMid:6827437.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013; 30(12): 2725-2729. http://dx.doi.org/10.1093/molbev/mst197. PMid:24132122.

Travassos L. *Serrasentis sagittifer* (Linton, 1889) (Acantocephala). *Mem Inst Oswaldo Cruz* 1966; 64(1): 1-10.

Williams H, Jones A. *Parasitic worms of fish*. London: Taylor Francys Ltd.; 1994.

Zhu X, Chilton NB, Jacobs DE, Boes J, Gasser RB. Characterisation of *Ascaris* from human and pig hosts by nuclear ribosomal DNA sequences. *Int J Parasitol* 1999; 29(3): 469-478. http://dx.doi.org/10.1016/S0020-7519(98)00226-4. PMid:10333331.